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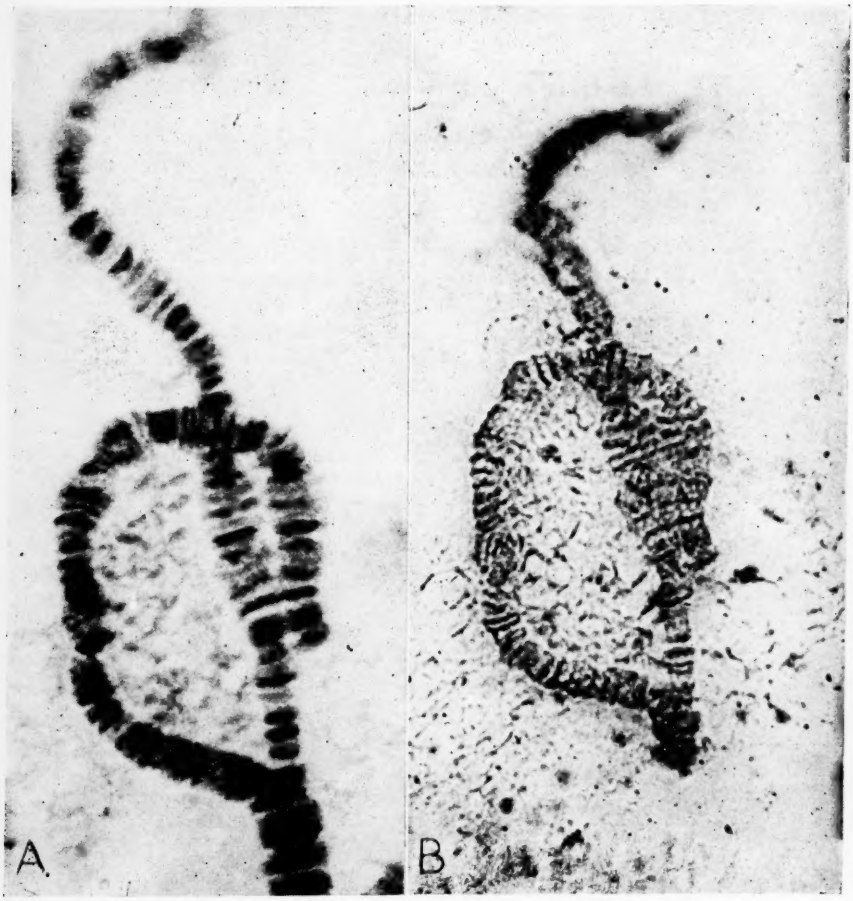

FRACTIONAL DIGESTION OF A CHROMOSOME
(SEE PAGE 235)

Hybrids Between Napier Grass and Millett

Crosseyed Cattle

Sesame Pollination

VOLUME 35 AUGUST, 1944 NUMBER 8

The American Genetic Association

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The

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TWO NAPIER GRASS HYBRIDS

Figure 1

These two very different looking plants are both first generation hybrids of Napier grass and cattail millet (*Pennisetum purpureum* × *P. glaucum*). That on the left is a chlorophyll deficient plant while the other is a plant with normal green foliage. These two types occurred among the F_1 hybrids in a ratio of 6 yellow plants to 10 green. The reason for such segregation among the hybrids of the first generation has not yet been determined.

HYBRIDS BETWEEN NAPIER GRASS AND CATTAIL MILLET*

GLENN W. BURTON†

NAPIER grass, *Pennisetum purpureum* Schumach, is a robust, non-saccharine bunch grass that reaches a height of 10 to 15 feet when mature. It was introduced from Africa in 1913 and, although it was soon planted in test plots on many farms in Florida, it never became an important economic species in that state. The common practice of propagating this bunch grass by the slow and laborious method of planting stem or crown cuttings no doubt restricted its use Susceptibility of the early introductions to the "eyespot" disease [caused by *Helminthosporium sacchari* (B. de H.) Butler[7] (*H. ocellum* Farris[8])], and improper management resulted in such poor stands and low yields that farmers soon lost interest in Napier grass. Blaser et al[1] have described management methods which make for increased production in this grass. Eyespot resistant strains developed by Ritchey and Stokes[5] in Florida and Burton[2] in Georgia have eliminated the disease problem mentioned above. At Tifton, Georgia, these eyespot resistant strains have survived temperatures of 12° F. while the eyespot susceptible strains have winterkilled badly at such temperatures. At Experiment, Georgia, eyespot resistant strains survived the comparatively mild winter of 1938-1939 but completely winterkilled in 1939-1940 when the temperature dropped to 7° F.

Cattail or pearl millet, *Pennisetum glaucum* (L.) R. Br., is a coarse annual bunch grass that reaches a height of 7 to 15 feet when mature. In Africa and Asia, where the seed are used for human food, this grass has been cultivated since prehistoric times. Cattail millet appears to be immune to the common grass diseases that occur in the Southeast and is unusually well adapted to the sandy soils of the Coastal Plain. It is readily established from seed and is widely used in several southeastern states as a soiling and temporary pasture plant. Experiments at the Georgia Coastal Plain Experiment Station[6] indicate that it will produce more beef per acre than other annual crops generally grazed in this area.

Hybridization

Some of the interspecific hybrids produced at Tifton, Georgia, by combining several species in the genus *Paspalum* have been described.[4] The study of the adaptability and productivity of these highly sterile hybrids suggested that interspecific hybridization might be successfully used to improve other species, particularly if the hybrids could be propagated vegetatively. Therefore, in the fall of 1941 an eyespot resistant clone of Napier grass was hybridized with two late maturing S_2 lines of cattail millet, using Napier grass as the female parent.

An estimated 2,000 mature spikelets of each hybrid were planted in flats of steam-sterilized soil in the greenhouse in the spring of 1942. Between 70 and 80 seedlings were obtained from each lot of seed and when three inches high were transplanted to small clay pots in the greenhouse. On May 20 these seedlings were planted in the field, spacing each individual four feet apart in rows six feet apart. Several rows of Napier grass seedlings, started and spaced in the same manner, were planted adjacent to the

*Cooperative investigations at Tifton, Georgia, of the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, the Georgia Coastal Plain Experiment Station, and the Georgia Experiment Station. The writer is indebted to Dr. O. E. Sell for information concerning the winter survival of Napier grass at Experiment, Ga.

†Geneticist, U. S. Department of Agriculture, Tifton, Georgia.

hybrid rows to permit a direct comparison between the species hybrids and Napier grass. There was no apparent difference in the size of these seedlings at the time that they were set in the field. Sixty-eight F_1 plants of the Napier × millet No. 805 cross and 66 F_1 plants of the Napier × millet No. 811 cross grew to maturity.

Vegetative Characteristics

The early growth habit of the Napier × cattail millet hybrids resembled that of Napier grass very closely. It was observed early in June that a number of the species hybrids were deficient in chlorophyll as if suffering from an extreme nitrogen deficiency. These yellow hybrids grew at a much slower rate than the green hybrid plants. A classification of these plants on October 1 revealed that 25 of the 68 Napier × millet No. 605 hybrids and 24 of the 66 Napier × millet No. 811 hybrids were chlorophyll deficient. The appearance of one of the largest of these chlorophyll deficient hybrids is shown in Figure 1. Most of these yellow plants were very small.

On November 25, green weights of a number of plants of Napier grass and Napier grass × cattail millet were determined. A summary of some of these results showed that 16 of the green Napier × millet No. 605 hybrids weighed an average of 29.82 pounds while 13 yellow hybrids of the same parentage averaged only 3.00 pounds per plant. The Napier grass plants that grew in the adjacent row averaged 14.24 pounds per plant. These yields indicate that many of the hybrid millet plants manifested considerable hybrid vigor.

On October 1, it was observed that four of the 43 green Napier × millet No. 805 hybrids and four of the 42 green Napier × millet No. 811 hybrids showed a very pronounced habit of branching at the nodes. The influence of this characteristic upon the appearance of the plant and individual stems is shown in Figures 2 and 3. The branching hybrid shown in Figure 2 appeared to be much leafier and outyielded all hybrid and Napier grass plants in this planting by a substantial margin, giving a green weight of 47.0 pounds on November 25, 1942. The two cattail millet parents, No. 805 and No. 811, showed very little tendency to branch at the nodes. Although the Napier grass parent, like most strains of this species, developed branches at the nodes of the main stems, they did not occur as early or as profusely as the branches on these eight hybrid plants. Many of the green hybrids demonstrated approximately the same degree of branching as the Napier grass parent while several, like cattail millet, produced very few branches. Morphological and cytological studies of these extreme types proved that all of them were hybrids.

Floral Characteristics

The genus *Pennisetum* is characterized by solitary or grouped spikelets surrounded by an involucre of bristles (sterile branchlets). These bristles occurred in about equal abundance in both parent species, but were about 50 per cent longer in Napier grass than in the cattail millet parents. At maturity the seeds (caryopses) of cattail millet are about half exposed while the seeds of Napier grass are completely enclosed within the glumes.

Representative heads of the Napier and cattail millet parents and of the green and yellow hybrids involving these species are shown in Figure 4. At first glance the heads of the green hybrids appeared to be very similar to the heads of Napier grass. More careful studies revealed, however, that these hybrid heads were longer, the spikelets were arranged more closely together, and the bristles tended to be shorter than similar organs on the Napier grass parent. Practically all of the florets in the green hybrid heads bloomed, exserting both stigmas and anthers. When compared with the heads of the green hybrids, the yellow hybrid heads were found to be much smaller and less compact. In the yellow hybrids the florets in the lower spikelets bloomed but the remainder of the florets were completely sterile.

Although the stigmas of the Napier ×

A VIGOROUS HYBRID COMPARED WITH NAPIER GRASS

Figure 2

The plant on the left is an unusually leafy hybrid of Napier grass and millet. That on the right is a plant of Napier grass. These plants were set out as seedlings on May 20, 1942 and this photograph was made October 5 of the same year. This plant is much more vigorous than the parent species and is very highly sterile. Thus it can be propagated as a forage plant without danger of becoming a weed as is the case with Napier grass.

cattail millet hybrids appeared to be quite normal the anthers were shriveled and failed to dehisce. Microscopic examination of these anthers revealed that they were completely empty or contained only a very few irregular pollen grains.

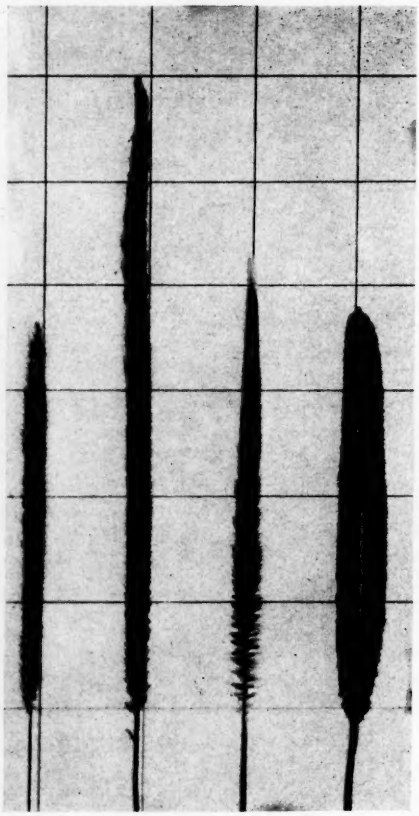
Fertility

In the fall of 1942 attempts were made to backcross four of the green Napier × cattail millet hybrids on both parents. Since the hybrids shed no pollen they were used as the female parent in all of the crosses attempted. A number of heads were also enclosed in bags in order to determine if any seed might be produced under bag without the application of pollen. Spikelets from these heads were planted in steam sterilized soil in the greenhouse in order to determine the number of viable seeds produced. No seedlings were obtained from heads enclosed in bags and not pollinated. Only one seedling plant was obtained from an estimated 5,000 hybrid florets that were pollinated with Napier grass pollen. Likewise, only one seedling was obtained from an estimated 5,000 hybrid florets that were pollinated with cattail millet pollen. These results indicate that these hybrids are very highly sterile. The two backcrossed plants obtained

HYBRID VIGOR

Figure 3

On the left is a stalk of the leafy Napier grass hybrid compared with the mother plant on the right. The green weight of the hybrid was a little better than double that of the Napier grass.



FLORAL CHARACTERISTICS

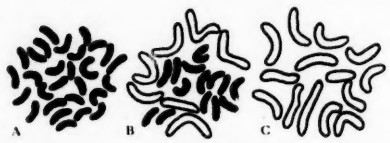
Figure 4

From left to right are representative heads of Napier grass, a Napier-millet with normal chlorophyll, and a hybrid with chlorophyll deficient foliage. On the right is normal cattail millet.

from these pollinations were different from, and less productive than, either the male or the female parents.

Cold Resistance

Temperatures of eight to ten degrees below freezing in December, 1942, killed the topgrowth of those hybrids and Napier grass plants that had not been cut for yield determinations. All topgrowth was removed soon thereafter. Unusually warm weather in January and early February of 1943 caused the Napier grass and the hybrids to resume growth and by mid-February growth had reached a height of from 6 to 18 inches. A week of minimum temperatures below freezing followed, with a low of 15° F. occurring on February 15. These temperatures killed all above-



CHROMOSOMES OF PARENT SPECIES AND HYBRID

Figure 5

Camera lucida drawings are shown of chromosomes at the equatorial plate in cells of root tips from (*A*) Napier grass, 2n = 28; *B* hybrid, *P. purpureum* × *glaucum*, 2n = 21; and *C P. glaucum*, 2n = 14. Chromosomes in the hybrid are inked as in the parent species to show their identity. There are 14 Napier grass chromosomes, and seven from the cattail millet parent, as would be expected.

ground growth of these plants and injured many other species that are usually not affected by such temperatures. Spring growth of both the Napier grass and of the millet hybrids was delayed. None of the Napier grass plants was killed, however, while 40 per cent of the hybrids, practically all of the yellow plants, failed to recover. Most of the surviving hybrids were less vigorous early in the spring and produced fewer shoots than the Napier grass plants. These results indicate that these Napier grass × cattail millet hybrids were not as cold resistant as Napier grass but were much more cold resistant than the annual cattail millet parent.

Cytological Investigations

Somatic chromosome numbers of 28 for Napier grass and 14 for cattail millet were reported by Burton[3] in 1942. It was also shown in this publication that the chromosomes of cattail millet were much larger than Napier grass chromosomes.

Cytological examination of the root tips of a number of the Napier × cattail millet hybrids considered here revealed that all of them possessed 21 somatic chromosomes. Representative equatorial plates of the two parents and one of the hybrids are shown in Figure 5. This figure demonstrates that it was comparatively easy on the basis of size and shape differences to identify the Napier and cattail millet chromosomes in the equatorial plates of the hybrids. No differences in number or appearance of the somatic chromosomes in the green and yellow hybrids could be found. It may be concluded on the basis of these studies that these hybrids are triploids.

Practical Considerations

Since these Napier × cattail millet hybrids can be established very readily from stem cuttings, the most common method used to propagate Napier grass, their sterility need not interfere with their usefulness. In some areas Napier grass has been considered a weed because its light, windblown seeds have germinated in nearby fields of other crops. In this respect the sterility of these hybrids would appear to be a desirable characteristic.

In some of the tropical countries, where freezing temperatures are never experienced, Napier grass is grown as a soiling or grazing crop and furnishes green feed for livestock throughout the year. Five of the best Napier grass × Cattail millet hybrids described here have been sent to several of these countries where their adaptability and usefulness will be studied.

The behavior of these hybrids at Tifton, Georgia, suggests they may be superior to Napier grass in the tropics.

Summary

The perennial Napier grass, *Pennisetum purpureum*, and two strains of late maturing cattail millet, *P. glaucum*, an annual, were hybridized using Napier grass as the female parent. These species hybridized readily and 134 F_1 hybrids were grown to maturity. Forty-nine of these hybrids were chlorophyll deficient, were about one-tenth the size of the green hybrids, and produced heads with a very high percentage of sterile florets. The green hybrids resembled Napier grass and many of them outyielded it materially. Eight of these green hybrids branched more profusely at the nodes than Napier grass while others, like cattail millet, branched very little. The heads of the green hybrids

produced stigmas and shriveled anthers which failed to dehisce and shed pollen. All of the hybrids were very highly sterile. Forty per cent of the Napier × cattail millet hybrids, including practically all of the yellow hybrids, and a few of the smallest green ones were killed by a minimum temperature of 15° F. Most of the surviving hybrids were less vigorous early in the spring and produced fewer shoots than the Napier grass plants, thus showing evidence of winter injury. Cytological examination of the root tips of a number of these hybrids revealed that all of them possessed 21 somatic chromosomes, the combined reduced numbers of the two parents. The identity of the Napier and cattail millet chromosomes in the equatorial plates of the hybrids was readily established. Five of the best hybrids appear to have considerable economic value and have been sent to several tropical countries for evaluation there.

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Size of Indianapolis Families

The present report is concerned with distribution of 6,551 native-white couples of virtually completed fertility (wife 40-44) according to number of live births. The analysis is carried through for Protestant and for Catholic groups separately by age of wife at marriage, monthly rental value of the dwelling unit, educational attainment, and birth region of the couple.

In the total sample, 18.8 per cent of the wives are childless and almost one-half (46.8 per cent) have one or two children, numbers below the average requirement for population maintenance. About 15 per cent have three children and 20 per cent have four or more.

The trend toward decreasing frequency of the large family is apparent from comparisons of the 1941 data with a somewhat similar sample previously extracted from the 1910 Census returns for Indianapolis.

By religion, the proportion childless is lowest (14.8 per cent) for the Catholic couples and highest (25.6 per cent) for the Protestant-Catholic mixed marriages. The proportion for the numerically predominant group of Protestant couples is 18.8 per cent, the same as that for the total group. The proportion of Catholic couples with five or more children is 17.1 per cent, as compared with 11.0 per cent for the Protestant couples and Protestant-Catholic mixed marriages.

With advancing age at marriage there is a sharp rise in the proportion of childless couples and an equally striking decline in the proportion with five or more children. Within the limits of the data available, this type of relation appears to be more pronounced among the Protestant than among the Catholic couples.

The relationship of family size to rental value and educational attainment is also sharper and somewhat more systematic among Protestant than among Catholic couples. With certain exceptions discussed in the report, however, the distributions exhibit the expected decreasing frequency of the large family with rising rental or educational status within each religious group considered.—C. V. Kiser and P. K. Whelpton. *Milbank Mem. Fund Quarterly*. January, 1944.

HEREDITARY STRABISMUS IN JERSEY CATTLE

W. M. Regan, P. W. Gregory and S. W. Mead
Division of Animal Husbandry, University of California, Davis

FIVE recessive hereditary defects have been reported by the authors from the Jersey herd of the California Experiment Station. All were brought to light by inbreeding. Two of these are lethals — namely, imperfect epithelium,[5] and recessive achondroplasia.[1] The other three are non-lethals —namely, a proportionate type of dwarfism,[2] congenital flexed pasterns[4] and congenital cataract.[3]

A sixth hereditary defect occurring in the herd is herein recorded. Animals manifesting a convergent type of strabismus, or crosseye, were first observed in 1928. No systematic record of the defect was initiated, however, until 1930, when all animals in the herd were examined and classified. Thereafter each calf was examined at birth and observed during the remainder of its life in the herd. Strabismus was not evident in any calf at birth. One animal was identified as defective at six months; all others were at least 12 months old when the anomaly was first observed. Not only are the eyes crossed, but they also protrude abnormally, both conditions being progressive in their development. The eyes of some of the mature cows became so severely crossed that vision was greatly impaired. Not all afflicted animals showed the character to the same degree; but those manifesting the defect in mild form were easily identified, provided classification was not attempted at too early an age. Even though the defect is undesirable and should be eliminated from a herd, some of the cows manifesting it to an extreme degree made exceedingly high records. At the time these animals were in the herd, a detailed study of the eye or eye muscles was not feasible.

The first affected animals were found in the inbred progeny of sire 300D, Pogis Torono Experimenter 177266, resulting from sire-daughter matings. Later, strabismus was found among inbred progeny of sire 3000, Lucky Fern 126758; and those of his son 320A, Rutgers Fern Napoleon 189646. Crosses made reciprocally by using 300D on daughters of 3000 (or 320A) and 320A on daughters of 300D also yielded animals with the defect. Sires 300D, 3000, and 320A all had normal eyes.

Table I summarizes the results of all the matings known to have produced strabismus animals. Many of the males were discarded at birth, before the eyes are visibly affected, and therefore could

TABLE I.—Summary of all matings that produced strabismus animals.

Sire	Cows	Progeny: Normal Female	Normal Male	Strabismus Female	Strabismus Male
300D	× own daughters	9	3	2	1
300D	× daughters of 3000 and 320A	16	5	2	1
320A	× own daughters and daughters of 3000	7	7	1	0
320A	× daughters of 300D	6	2	1	0
428A (normal son of 320A)	× daughters of all heterozygous sires	3	1	1	0
Total		41	18	7	2

Analysis of females	Normal	Strabismus
Observed	41	7
Expected	42	6
d	−1	+1

Analysis of males		
Observed	18	2
Expected	17.5	2.5
d	+.5	−.5

Males and females combined without taking the sex ratio into account		
Observed	59	9
Expected	59.5	8.5
d	+.5	−.5

not be classified. This fact accounts for the limited number of males.

The expected results are calculated on the basis that one autosomal recessive gene conditions strabismus and that the original cows were homozygous normal. Since less than half the males were classified, females and males are analyzed separately and then combined without taking the sex ratio into account.

All the observed results agree closely with the theoretical expectations. Apparently, therefore, strabismus is conditioned by one autosomal recessive gene.

The use of homozygous normal bulls promptly stopped the occurrence of crosseyed animals.

The gene for strabismus was introduced into the herd by bulls 300D and 3000. As their pedigrees show, they have no common ancestors in the first four generations. Farther back, however, they have some. The data do not indicate whether these two bulls obtained the gene for strabismus from the same ancestral source or as a result of two independent mutations. Judging from our experience in analyzing other mutant genes in the herd, origin from a common ancestor is more likely.

Summary

Several animals manifesting convergent strabismus were found in the California Experiment Station herd. All individuals that became affected had normal eyes at birth, the eyes becoming crossed in varying degrees at six to twelve months of age. The eyes of some of the mature animals became severely crossed. Strabismus is inherited and is conditioned by a single recessive autosomal gene. Two relatively unrelated bulls introduced the gene into the herd. The use of homozygous normal bulls promptly stopped the occurrence of strabismus animals.

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Sex Hormones in Protozoa

Kimball (*Genetics* 27:269) therefore proposes the hypothesis that each of the three mating type genes mt^1, mt^2 and mt^3 [in Euplotes] controls the secretion of a different substance which is capable of inducing conjugation among animals of mating types that do not secrete the same substance. In support of this hypothesis, Kimball presents evidence that in mixtures between mating types fewer pairs of conjugants form when one type is heterozygous and the other type is homozygous for one of its alleles than form in other combinations. This result agrees with the hypothesis, for in such mixtures the fluid from the homozygous race will not induce animals of the heterozygous race to mate with each other, while in all other combinations there may and probably do occur all three possible kinds of matings among the two mating types represented.

This work of Kimballs is the first and at present only case of diffusible sex substances in Protozoa and appears to provide a very favorable opportunity for study of the sexual substances from both developmental and genetic points of view. Here then is an opportunity to compare the phenomena in a unicellular animal with those set forth by Kuhn and Moewus for the unicellular plant, Chlamydomanas. Both for this reason and because of its obvious important possibilities, the further development of Kimball's researches will be awaited with much interest.—T. M. Sonneborn. *Cold Spring Harbor Symposia* 10:124.

STUDY OF FINE CHROMOSOME STRUCTURE UNDER ENZYME TREATMENT

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THE chromonema theory is at present the principal morphological theory of chromosome structure. The chromonema is usually now defined by Nebel's[16] description as the "optical single thread within the chromosome" which modifies the original concept of this term as of a thread staining wtih basic stains. On the basis of this explanation of the chromonema, Kuwada[14] writes that the chromonema is the only component of the chromosome which retains its individuality throughout the chromosome cycle and which is therefore autonomous even at the stage of the interkinetic or resting nucleus.

Actually during the transition from the telophase to the stage of the "resting" nucleus the chromonemata undergo marked changes. They lose their thymonucleic acid and therefore they do not stain with basic stains and do not give a positive Feulgen reaction. The nucleolus is formed from the proteins of the chromosomes, viz., from those of the chromonemata,[2] and the karyoplasm becomes rich in proteins. Therefore it is not the chromonemata, but their protein framework, the genonemata, which are retained. According to the hypothesis first proposed by N. K. Koltzoff[10] and accepted by Wrinch,[19] Frey-Wissling,[6] Caspersson,[2,3] Schmidt[17] and others, the protein framework of the chromonema (the genonema), is formed of a bundle of giant chain molecules such as are found in silk fibroin, keratin, myosin, etc. Side protein chains or genes are postulated by Koltzoff[12] to adjoin the backbone of these chain molecules. Given a considerable length of the genonemata, it is assumed[12,16] that their diameter is below that of optical resolution.

The genonemata are assumed to become optically visible owing to a surrounding layer of material whose precise chemical nature is unknown. This was termed "chromoplasm" by N. K. Koltzoff, and "matrix" by many authors. A synthesis of nucleoproteins (chromatin) takes place at prophase around definite regions of the genonema (genes); in this way are formed the chromatin chromomeres. Beginning with this period and continuing into the telephase one may speak of the chromonemata. The problem of the number of chromonemata in the chromosome has not been solved. According to Darlington the anaphase chromosome has only one thread, according to a majority of authors, two; and according to Nebel there are four threads.

Each chromosome is conceived to be formed of two intertwisted chromatids. Each chromatid consists in turn of two chromonemata. Preparations of *Vicia faba* made by the author confirm Nebel's theory of the existence of four chromonemata in the anaphase chromosomes. The prophase chromosome of the next mitosis must also consist of four chromonemata. However, when the intertwisted threads are strongly stretched, the secondary chromonemata come into closer contact and cannot be optically distinguished. Thus, at the early prophase usually only two chromonemata are visible, although actually there are four. At prophase, doubling of the genonemata takes place while at the late prophase four chromonemata (though in reality there are eight) may be distinguished. At metaphase each chromosome half clearly consists of two intertwisted chromatids. However, owing to the close contact of the chromonemata

*Dedicated to the memory of my teacher, N. K. Koltzoff.

NUCLEASE TREATMENT OF CHIRONOMUS CHROMOSOMES

Figure 6

The same chromosome is shown as initially stained with aceto-carmin (*A*), after nuclease treatment (*B*), and after subsequent hydrolysis and determination of a negative Feulgen reaction (*C*). The treatment with nucleases assumedly completely decomposes the nucleic acids, as was demonstrated by a negative Feulgen reaction. Hydrolysis did not produce any visible changes in the residual protein structure.

the duality of each chromatid is rarely visible and becomes manifested only at late anaphase or telophase.*

The facts above mentioned show that there is in reality no difference between the structure of the chromosomes at mitosis and those of the salivary glands.[7] In both cases the chromosomes are

*Upon another fixation and staining the spaces between two points where two chromatids or chromonemata cross each other give the impression of large chromomeres which are frequently described at this stage.[7,18]

formed of intertwisted chromonemata.*

It is difficult to elucidate this structure on the metaphase chromosomes, though in good aceto-carmin preparations it is visible.

Study of Chromosome Structure by Means of Nuclease Treatment

The continuity of the protein framework of the chromonemata is proved by Casperson[1,2] and Mazia and Jaeger.[15] The latter removed the thymonucleic acid by means of enzymes (nucleases); next they applied the ninhydrin reaction for protein and the uninterrupted protein framework of the chromosome stained blue. No thickenings, corresponding to chromomeres, were found by Mazia and Jaeger in the protein threads; therefore the problem of the structure of the chromomeres remained unsolved.

I modified the method of Mazia and Jaeger and used sections of salivary glands fixed with alcohol and aceto-carmin preparations, instead of live salivary glands. I did not succeed in obtaining an active nuclease (solution) from spleen juice prepared by van Herwerden's techniques as applied by Mazia and Jaeger. I used pure tissue juice pressed from beef spleen and diluted with water. This tissue juice was obtained in two ways: (1) the juice was pressed from rubbed spleen by means of Buchner's press and was subsequently diluted with water and centrifuged. After addition of chloroform the juice may be preserved for a long time in the refrigerator. Chloroform contributes to protein precipitation, especially at 37°, while the enzymes remain in the solution; (2) the second method was developed by M. A. Peshoff, and will not be detailed here.

It was assumed that this juice contained all the enzymes known under the term of nucleases: *polynucleotidases* (splitting polynucleotides into mononucleotides), *nucleotidases* (splitting off phosphoric acid in the formation of nucleosides), and *nucleosidases,* splitting off nitrogenous bases and thus completing the decomposition of nucleic acids.

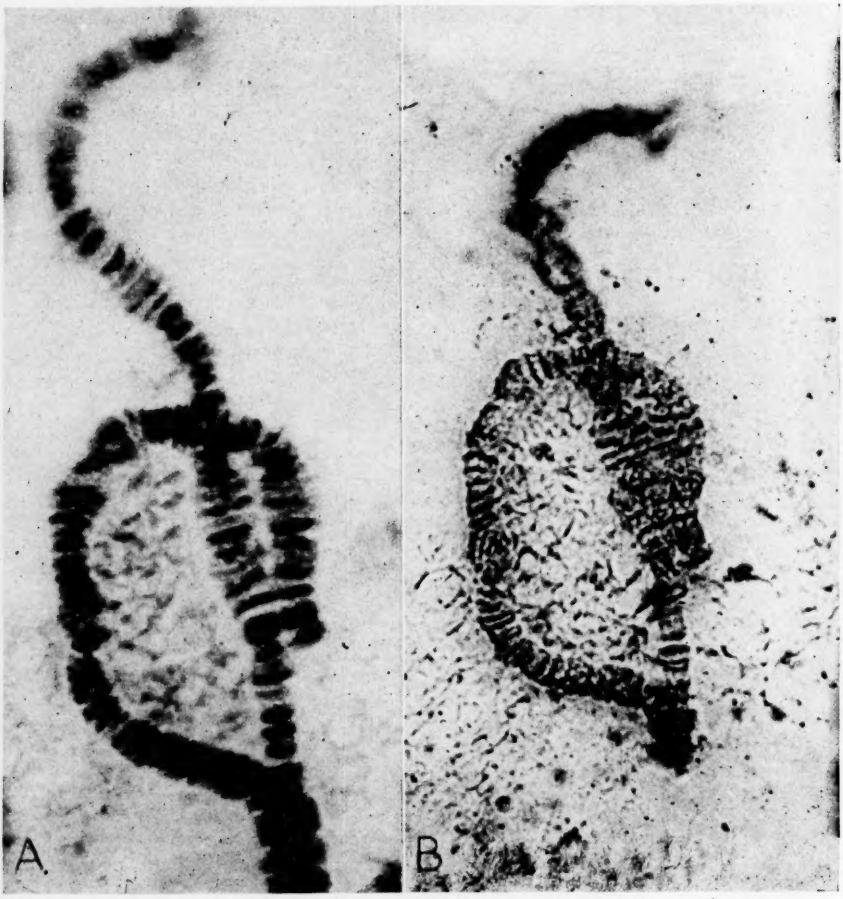
The chromosome preparations, placed in the spleen juice-chloroform reagent, were incubated at 37°, until a negative Feulgen reaction indicated that all thymonucleic acid had been decomposed. In order to check the action of the enzymes the preparations were transferred into boiled juice. The Feulgen reaction was controlled by aceto-carmin preparations which had not been treated with nucleases.

The time necessary for the removal of the thymonucleic acid, as indicated by a negative Feulgen reaction, served as criterion for the activity of the enzymes of the juice. The enzymes are most active in a neutral medium (pH 7). If the juice was acid, a neutral reaction was established by the addition of 1/10 N NaOH or by dilution with phosphate buffers, the former giving the best results.

In order to obtain a negative Feulgen reaction with *Drosophila* chromosomes, digestion for at least 20 hours was required, and *Chironomus* chromosomes required even longer. Prolonged immersion in the juice (for several days) gave equally satisfactory results. After this had been established I kept the *Drosophila* preparations in the spleen juice for 36 hours and those of *Chironomus* for 48 hours to be certain that the thymonucleic acid was entirely removed.

After treatment with nucleases and checking with the Feulgen reaction, the preparations were examined in glycerin or glycerin-gelatine (in Canada balsam chromosomes thus processed are so transparent that they can scarcely be distinguished). In glycerin the entire structure of the completely colorless chromosomes becomes visible before the action of the enzymes can be seen. The separate chromomeres, as well as the bands, stand out clearly both in Figures 6*A* and 6*B*, while the genonemes situated between the chromomeres are more dis-

*The number of chromonemata amounts to 16 in the chromosome of *Drosophila* and may be above 200 in *Chironomus*.

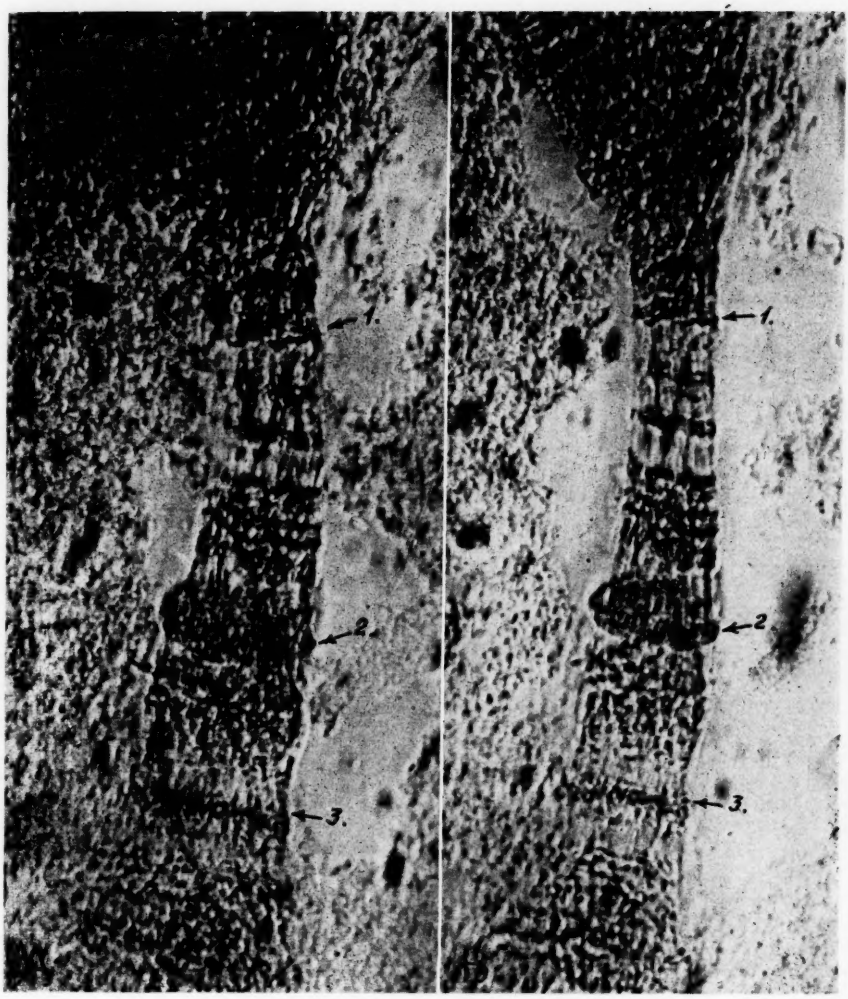


REDUCTION IN SIZE

Figure 7

Chromosome II of *Drosophila robusta* containing an inversion (loop). *A* shows an aceto-carmin preparation before treatment. *B* shows the same preparation after nuclease treatment. Note the definite reduction in size. This sometimes occurred but not always when chromosomes were exposed to enzymes which digested the nucleic acid.

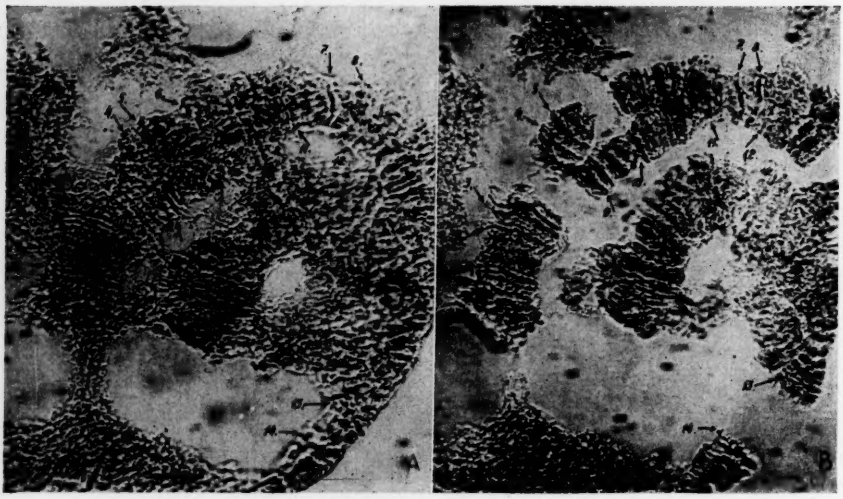
tinct than before the experiment, as if something had been removed from between them. That this actually has happened is suggested by the fact that a partial dissolution also occurs in the cytoplasm of the salivary gland cells surrounding the chromosomes on the aceto-carmin preparations (compare structure of cytoplasm on the three photomicrographs of Figure 6). This is probably due to a splitting by the nucleases of the ribosenucleotides which are present in the cell plasm and apparently also in the chromosomes between the chromonemata. In order to answer the question whether a reduction of chromomere size accompanies the removal of the polynucleotides, the chromosomes were measured on the photomicrographs. After the action of the nucleases (Figure 6*B*)



RESULT OF PEPSIN TREATMENT

Figure 8

A is an aceto-carmin preparation of a *Chironomus* chromosome before treatment. *B* shows the same preparation after 25 minutes' treatment with a one-half percent solution of pepsin. The pepsin dissolves the proteins and results in slight reduction of size of the chromosome and in increased transparency.



BREAKS CAUSED BY PEPSIN TREATMENT

Figure 9

A shows an aceto-carmin preparation of *Chironomus* salivary gland chromosome before treatment. *B* shows the same chromosomes after 25 minutes treatment with dilute pepsin. The arrows indicate identical areas in the two figures. Breaks indicate that there has been a considerable reduction in size. Digestion of the proteins has brought out more clearly the chromosome structure.

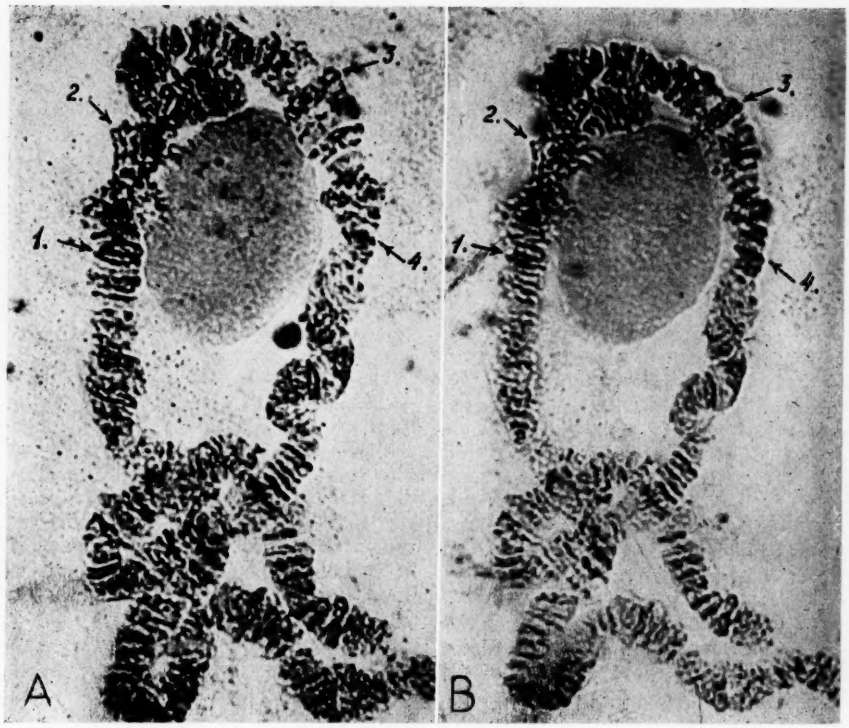
a slight reduction of chromosome length takes place as compared with the aceto-carmin preparation (Figure 6*A*); however no reduction of chromomere size was found. Subsequent hydrolysis with hydrochloric acid (Figure 6*C*) did not produce any changes.

In some cases, upon complete preservation of the structure, a considerable reduction of chromosome size was observed (Figure 7) which might have been caused both by the action of enzymes and by hydrolysis; the latter occasionally causes a strong reduction in size, especially in plant cells. The reduction of the size of the entire chromosome naturally affects the relative reduction of chromomere size. On the other hand, cases of a considerable increase of chromosome size under the effect of enzymes of spleen juice were observed (swelling) and after HCl hydrolysis the size was not reduced.

The results obtained on salivary glands were verified on other objects Complete preservation of the structures was observed at all growth stages of the spermatocytes of the grasshopper; after treatment with nucleases the chromomeres are more clearly visible than prior to their action. The metaphase chromosomes in the spermatogonia of the grasshopper and in the root cells of *Vicia faba* did not diminish under the action of nucleases. Occasionally swelling was observed.

After a negative Feulgen reaction, preparations were stained with a number of basic and acid stains. Borax carmin, soluidin blue, methylene green and haematoxylin gave negative results. When the preparations are kept for a long time in Karazzi's haematoxylin, a diffuse staining of the entire preparation, of the chromomeres, genonemes and surrounding cytoplasm, is observed. The chromosomes stain well with acid stains: light green and acid fuchsin. Acid fuchsin stains the chromosomes and the surrounding cytoplasm very rapidly and intensely.

The negative Feulgen reaction and



PEPSIN TREATMENT OF DROSOPHILA SALIVARY GLAND CHROMOSOME

Figure 10

A shows an aceto-carmin preparation of an X-chromosome of *Drosophila robusta*. Arrows on both figures denote corresponding regions. Arrow *2* marks the inert region of the double armed X-chromosome connected with the nucleolus. Pepsin treatment has resulted in a reduction in size and a slight increase in the clarity of outline.

the reaction of the chromosome to basic and acid stains, speak for the complete splitting of the polynucleotides. The nucleosidases complete the disintegration of the nucleic acids, splitting off nitrogen bases only after the action of polynucleotidases and nucleotidases, viz. after the nucleic acid has been split off from the protein. All the disintegration products of the polynucleotides are soluble and do not remain in the chromosome.

The final proof that the thymonucleic acid had been completely removed might be obtained by obtaining the ultraviolet absorption spectra of these chromosomes. The absence of an absorption at 2,600 Angstroms, the point of maximum absorption by nucleic acids, might be a conclusive proof of the splitting off of all thymonucleic acid. The absence of thymonucleic acids might allow a more accurate study of the protein composition of the chromosomes than so far has been done by Caspersson[2] in the presence of thymonucleic acids. However, owing to the absence of necessary apparatus other ways for establishing the entire absence of thymonucleic acid had to be sought for.

Pepsin Treatment

I concentrated my studies on the action of pepsin on the chromosomes in

connection with the prevailing viewpoint that thymonucleic acid preserves the nucleotides from being digested by pepsinase. Although Fruton and Bergmann[9] published data indicating that pepsin may destroy some peptid links, nevertheless Nazia and Jaeger[15] demonstrated that the integrity of the salivary gland chromosomes is preserved after the action of pepsin.

My work with pepsin treatment was started in December, 1940. Owing to lack of crystallized pepsin I used pepsin of the Merck firm. I applied a 0.5 per cent solution of pepsin, with HCl to bring the pH to 1.94.

The experiments with pepsin digestion were carried on in permanent aceto-carmin preparations of salivary gland chromosomes of *Drosophila* and *Chironomus* larvae, both with those not subjected to the action of nucleases, and after the removal of thymonucleic acid. The *Chironomus* preparations were especially convenient owing to the large size of the chromosomes. The preparations were placed in a petri dish upside down on the heating stage of the microscope and the digestion process was observed at 37° under the microscope. For prolonged pepsin action the preparation was frequently immersed in a vial containing pepsin solution and was kept in an incubator at 37-39°.

Action of Pepsin on Aceto-carmine Preparations of Salivary Gland Chromosomes

Chromosomes not subjected to the action of nucleases sometimes swell slightly when water is replaced by pepsin, however, in a few minutes the outlines of the structure are reestablished. In about ten minutes bands become visible on the thick, slightly elongated chromosomes of *Chironomus* which were so far invisible, while the solid bands break into separate chromomeres. The chromosomes become gradually more transparent (Figure 8). At the same time dissolution of the cell cytoplasm of the salivary gland chromosomes takes place. This may be related to the increase in transparency of the chromosomes and may be explained by dissolution of the proteins filling up the spaces between the chromonemata. These simultaneous processes suggest that there is no essential difference between the chemical composition of the cytoplasm of the salivary gland cells and the substances filling up the spaces between the chromonemata. These substances may be, therefore, designated as the plasm of the chromosome, namely *chromoplasm.*

The salivary gland chromosomes of *Drosophila* contain few chromonemata. Hence the removal of a certain amount of chromoplasm by the action of pepsin does not greatly increase the transparency of the chromosomes (Figure 10). A reduction of chromosome size takes place simultaneously with the increase in transparency. It occurs in all directions and may cause mechanical breaks. It becomes visible about 15-20 minutes after the chromosomes were placed into heated pepsin solution. (cf. Figure 9.) A prolonged treatment with pepsin (24 hours) causes further shrinkage and increases the number of breaks. Chromosome contraction has a definite limit, and further treatment with pepsin (up to five days) is not accompanied by an increase of breaks.

Action of Pepsin on the Salivary Gland Chromosomes After Treatment With Nucleases

The result of the action of pepsin depends on how long the preparations were acted on by the spleen juice nucleases. If the latter were feebly active or if the preparation was subjected to their action so short a time as to yield a feeble positive Feulgen reaction, then the action of pepsin in these chromosomes will not particularly differ from that described for the chromosomes not subjected to the action of nucleases. In this case the chromosomes become increasingly transparent, as destruction and washing off of a large amount of chromoplasm between the chromonemata takes place. Simultaneously the cytoplasm of the salivary gland chromosomes is dissolved entirely, whereas on the preparations not subjected to action of nucleases it is dissolved only partially during the same space of time. I suggest that the preliminary splitting up of ribosenucleotides

by nucleases affects the dissolution rate of pepsin on the cytoplasm and the chromoplasm.

The action of pepsin produces different results if the chromosomes give a negative Feulgen reaction. When the water is replaced by heated pepsin the structures swell to a certain extent and their outlines become temporarily less pronounced. At the same time the chromoplasm between the chromonemata is dissolved, the duration of the first period depending on that of the action of the nucleases (from 15 to 30 minutes). Next, the bands and separate chromomeres begin to increase greatly in size, viz. to swell, and the space between them deepens (second period lasting up to five minutes); immediately after this strong swelling sets in, the structures become invisible (third period) and in five minutes digestion begins (fourth period). In five to ten minutes rows of minute chromomeres are visible instead of the chromosomes; sometimes fine longitudinal threads uniting these chromomeres may be discerned. I think that these fine threads with thickenings instead of the large chromomeres are genonemata. They do not change under a prolonged action of pepsin (24-28 hours) and they do not stain with acid fuchsin.

If the preparation is kept in spleen juice for some time after the thymonucleic acid has been removed, viz. after the chromosomes give a negative Feulgen reaction, the rate of pepsin action on the chromosomes increases. The first period seems to be omitted; as soon as pepsin is added a strong swelling sets in, the structures become almost invisible, and rapid dissolution begins. Two to three minutes after the pepsin is added nothing remains of the chromosomes.

Sometimes the process proceeds more slowly and it lasts for about 15 minutes. In this case it is possible to observe clearly that after marked swelling the bands or separate chromomeres begin to be gradually visible. The spaces between them deepen; next, the structures begin to grow fainter, digestion sets in and is complete in from two to three minutes: the chromosome entirely disappears.

The process of the complete digestion of the chromosomes by pepsin was cinematographed. The entire process lasted five minutes. Separate stages showing the succession of this process are given in Figure 11. On the last photomicrograph (Figure 11*l*) some chromosome particles are preserved. At this stage cinematographing was stopped.

Not all parts of the chromosome are digested at the same rate. Sometimes only part of the chromosome undergoes complete digestion while in the other parts which were either covered by the cytoplasm, or less stretched, the abovementioned minute chromomeres are retained. On the same preparation some chromosomes may be completely digested while others retain a skeleton of fine structured genonemata.

Conclusions and Discussion

The morphological data of this investigation on chromosome structure during mitosis are in agreement with my deductions on the structure of the salivary gland chromosomes.[7] The chromosome consists of chromonemata successively twisted into pairs. The difference between the salivary gland chromosomes and ordinary mitotic chromosomes consists merely in the length of the chromonemata and in the compactness of the coils.

The experiments with enzyme treatment prove that the spaces between the chromonemata are filled with a substance which I consider more correct to term according to N. K. Koltzoff as chromoplasm, viz. plasm of the chromosome (not matrix), since its chemical composition approaches that of the cytoplasm or karyoplasm. This conclusion on the chemical composition is based on the similarity of action of enzymes, viz. nucleases and pepsin, on the chromoplasm of salivary gland chromosomes and on the surrounding cytoplasm of the cell. The partial digestion of the cytoplasm and chromoplasm by nucleases speaks for the presence of ribosenucleotides in both these, while the more rapid and complete digestion of the proteins by pepsin after removal of the ribose-

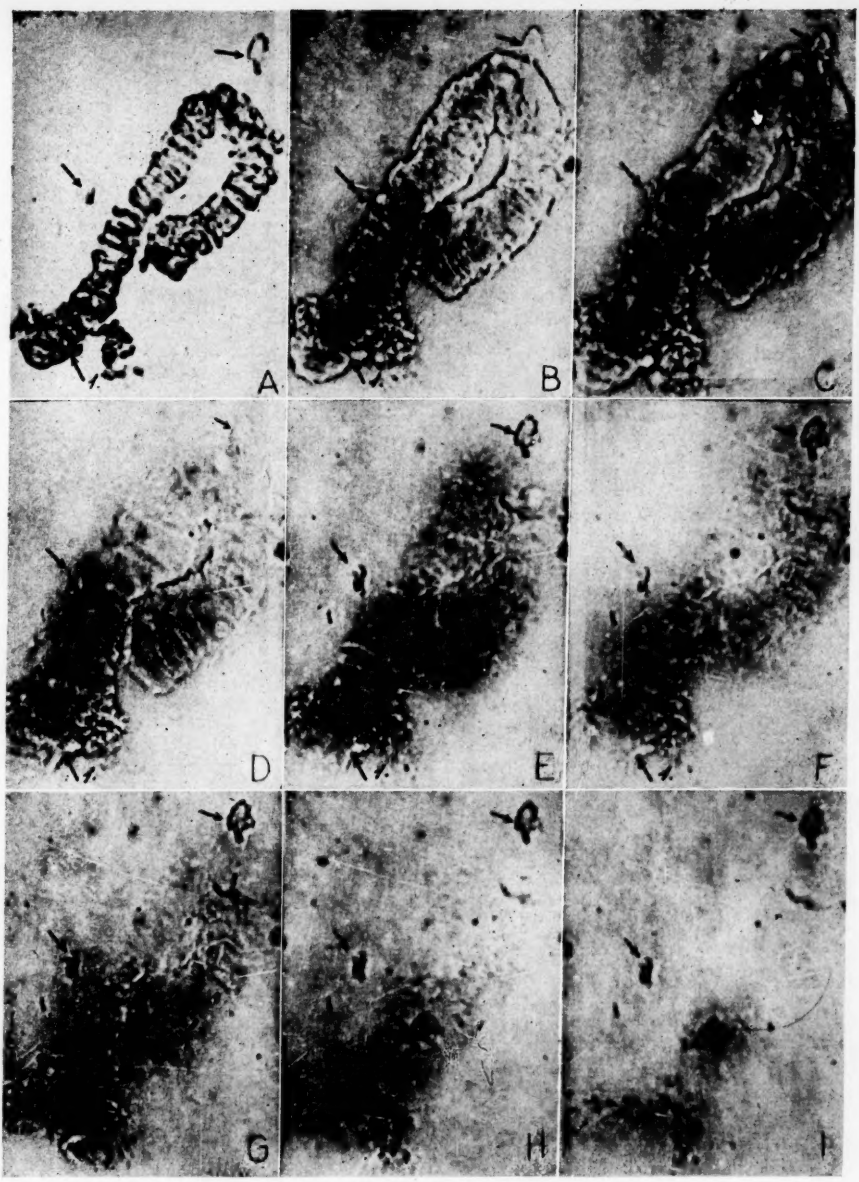
nucleotides by nuclease action speaks for the presence of the same kind of proteins in both these structures.

Each chromonema is formed by an alternation of chromomeres giving a positive Feulgen reaction and of the spaces between them. The complex of enzymes from spleen juice termed nucleases causes an entire splitting up of polynucleotides, the chromosomes do not stain with Feulgen, while their shape and size are retained. The experiments with pepsin showed that the negative Feulgen reaction may be considered as proof that the thymonucleic acid is entirely removed from the chromosome. Everything that remains after the action of nucleases should be regarded as the protein framework of the chromosomes. This fact confirms once more the continuity of the protein framework of the chromonemata whose pairs twisted about each other form the chromosomes, and to a certain extent explains the structure of the chromomeres. The chromomeres have a protein framework which undergoes fixation and is not affected by the enzymes from spleen juice. The polynucleotides join the protein molecules both without and within the chromomeres in the spaces between the polypeptides, owing to this the chromomeres did not visibly change in size after removal of the polynucleotides. This explanation is in agreement with the data of Caspersson[3] on the absence in the chromomeres of a regular orientation of the molecules of the thymonucleic acid.

My experiments on the digestion of the chromosomes by pepsin prove that the protein framework of the chromonema remaining after the action of nucleases cannot be considered as a genonema. The genonema becomes visible only after pepsin has digested the protein sheath enveloping the genonema. According to Caspersson these proteins are histones in the chromomeres which had split off polynucleotides under the action of nucleases and proteins of globulin types in the spaces between the chromomeres. After the protein sheath had been digested a number of minute chromomeres joined by fine threads were found in many preparations of the salivary gland chromosomes of *Chironomus*. They do not undergo further digestion by pepsin and do not stain with acid stains. These structures are on the limits of vision and I did not succeed in taking good photomicrographs of them. These fine threads with minute chromomeres (genes) confirm the hypothesis of N. K. Koltzoff on the structure of the genonemata and I believe that they must be regarded as genonemata.

Upon transition from the telophase to the stage of the "resting" nucleus the chromosomes cease to be stained by Feulgen because destruction of the nucleoproteins takes place. According to Caspersson the nucleoli are formed from the histones, while part of the histones together with the proteins of a higher type remain in the karyoplasm. In other words, the genonemata, which have lost their external sheath of nucleoproteins (chromatin) and are surrounded only by a layer of chromoplasm remain in the interkinetic nucleus. It is possible that in for "resting" nuclei of different organisms the genonemata are surrounded by an unequal layer of chromoplasm. In some nuclei the genonemata are easily visible, retaining the arrangement of the chromonemata at telophase.[5,8] In some optically empty nuclei no structures are found. The assumption is not excluded that in the latter the layer of chromoplasm surrounding the genonemata is so fine that the dimensions of the diameter of the threads cannot be optically discerned. A synthesis of nucleoproteins around the minute thickenings on the genonemata (genes) takes place at prophase; with other words, the chromatin chromomeres are formed, giving the positive Feulgen reaction.

The experiments on the dissolution of the chromosome protein framework have shown that after the action of enzymes from spleen juice, pepsin destroys the peptide links which are usually not affected by it. This is undoubtedly connected with the splitting off of polynucleotides from the protein molecules (histones) in the chromomeres. The genonemata may in their turn be digest-



COMBINED NUCLEASE AND PEPSIN TREATMENT

Figure 11

These nine "frames" from a motion picture film show the complete digestion of a chromosome first treated with nucleases until a negative Feulgen reaction showed that all nucleic acid had been removed. The "residue" was then stained with aceto-carmin (*A*). *B* shows the same chromosome immediately after immersion in a dilute (.5%) solution of pepsin at 37 degrees. The other photographs show progressive stages in the complete digestion of the chromosome. At first (*D*—*E*) there is an increase in the clarity of separate bands, as the proteins begin to be digested. At *I*, the end of the film, only a few particles within the chromosome have remained undigested. Arrow "1" (in *G*) denotes one such region. The two other arrows indicate spots lying outside the chromosome.

ed by pepsin if the chromosomes continue to be treated with enzymes from spleen juice after the thymonucleic acid is removed. In this case nothing remains of the chromosomes. It is possible that besides splitting off the polynucleotides some other groups (possibly amino groups) split off from the protein molecules, causing changes in the stability of the peptide links in respect to pepsin. It is probable that this splitting off is caused by other than nuclease enzymes from spleen juice, and that it may take place only after the removal of polynucleotides, since the genonemata are digested only if the chromosomes were subjected to the action of enzyme from spleen juice for some time after all thymonucleic acid was removed. One can safely say that tripsinase is absent here, since the chromosomes are not digested in alkaline spleen juice (pH 8.3). This complete digestion of the entire protein framework of the chromosomes may possibly be explained by the destruction of only some peptide links; however, owing to this the chromosomes break into minute particles which are below optical resolution and both visually and on the photomicrograph one has the impression that they are entirely lysed.

My explanations of the observed digesting action of pepsin are hypothetical. Further experiments preferably with pure enzymes are necessary.

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Tri-Racial South Africa

The population of the Union of South Africa consists of three main racial groups, plus a fourth of mixed racial origin. The total population in 1936 was 9,590 thousand; of these, 21 per cent were European, 69 per cent native, 2 per cent Asiatic, and 8 per cent mixed and other non-European, primarily the Cape Coloured. Natives constitute the majority of the population of all areas; 58 per cent in the Cape (including the Transkeian Territories), 80 per cent in Natal (including Zululand), 73 per cent in the Transvaal, and 72 per cent in the Orange Free State. Europeans formed less than 10 per cent of the total population in 81 of the 249 magisterial districts into which the Union is divided, and less than 25 per cent in 127 districts. In only nine districts did they form 50 per cent of the total population, and in none did they exceed 60 per cent. Europeans were the most urban of all groups, with 33 per cent living in cities of 100,000 or over as contrasted with 14 per cent for the total population. Conversely, the proportion rural was 34 for the European, 68 for the total population.—*Population Index.*

"COMPACT" SHORTHORNS*

H. H. Stonaker and R. C. Tom†

An extreme degree of compactness in body conformation has been observed in three purebred herds of Shorthorn beef cattle located in Colorado, Nebraska, and Kansas. Within these herds the variation in body type or conformation is not of a continuous nature but is broken into two easily distinguishable populations—a group of small, very compact, short-legged, thick animals and another group of larger more upstanding animals that are representative of the conformation found in many purebred Shorthorn herds.

Two genes have previously been reported which have a somewhat similar effect on body type in cattle. Lush‡ reported a group of extremely short-legged cattle in a range herd of Herefords. He attributed the condition to the action of a dominant gene. A dominant lethal gene in Dexter cattle has long been known which in the heterozygous state causes an extreme degree of compactness and short-leggedness. When homozygous, this factor is lethal, causing the development of "bulldog" calves which are either aborted or born dead.

The "compact" type found in these Shorthorn herds can be identified at birth and is distinct throughout the life of the animals. They appear shorter in head, neck, body, and legs than the "standard" type of Shorthorns. Although their weights have not been systematically recorded, they appear smaller in size at all ages. They also seem to be somewhat thicker in their fleshing than the "standard" cattle. Some of the animals have a tendency to be heavy in the shoulders and a bit crooked in the legs. Because of their extreme beef conformation, these cattle have found favor with several purebred and commercial breeders and have been popular for the production of fat show steers.

Origin and Distribution

The Kuhrt family near Edson, Nebraska, was one of the first to begin concentrating the blood of this type in their herd, so far as the authors have been able to discover. The first bull of this type was used in their herd in 1933. He was Rosewood Gift 1696585, bred by Johnson and Ault.

At the present time the Kuhrts are getting calves from the first generation of "compact" females bred back to "compact" bulls. The "compact" bull, Victor's Gift, bred by the Kuhrts was also used in the Crews & Crews herd at Haigler, Nebraska, and "compact" sons bred by Crews & Crews have been used in the Lindgren herd, Wray, Colorado, and in the Colorado State College herd, Fort Collins, Colorado.

Inheritance of the "Compact" Type

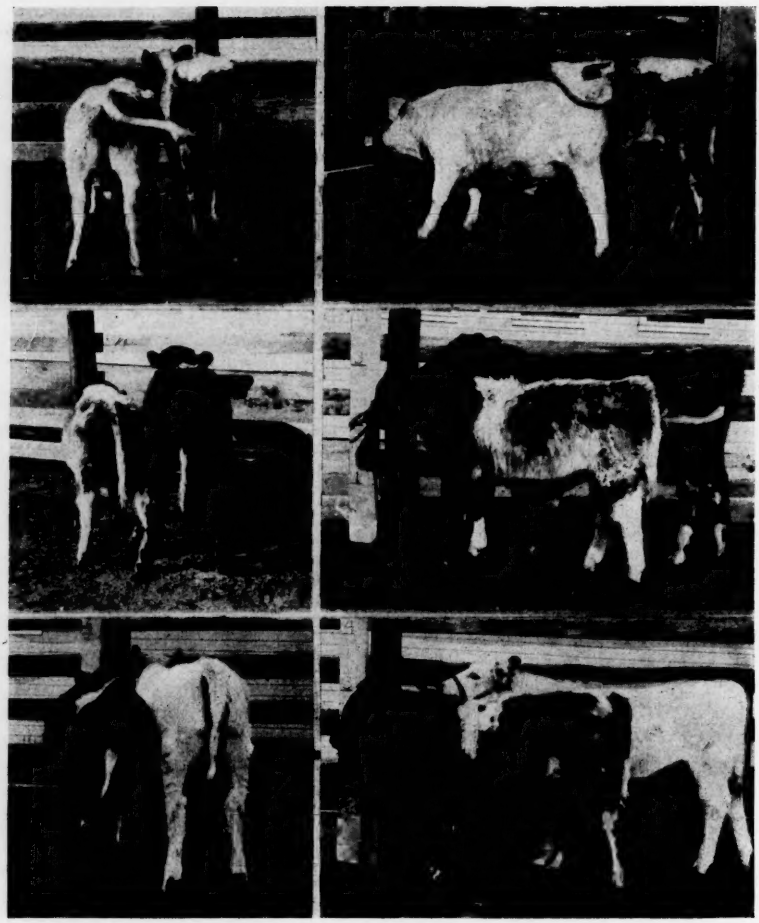
In the fall of 1943 unselected calf crops of the Kuhrt, Crews, and Lindgren herds were divided according to their type. In making this classification there were but a few instances where it was difficult to tell in which group the calf belonged. In the Crews and Lindgren herds all the calves were out of "standard" type cows; in the Kuhrt herd 27 of the cows were of the "compact" type and 20 were of the "standard" type. In all three herds the bulls used were of the "compact" type. These bulls were in turn sired by "compact" type bulls and out of "standard" type cows.

The numbers of "compact" and "standard" calves found in the three herds are reported in Table I.

*Scientific series paper No. 170, Colorado Agricultural Station.

†Associate professor, on military leave; and professor and head, respectively, in Section of Animal Investigation, Colorado Agricultural Experiment Station, Colorado State College.

‡Lush, J. L. "Duck-legged" cattle on Texas ranches. Jour. Hered., 21:84-90. 1930.



"COMPACT" AND "STANDARD" STEERS

Figure 12

Pairs of "compact" and "standard" type steer calves at six months of age from the Lindgren, Colorado State College, and Crews herds (top to bottom). The pairs are sired by three different "compact" bulls and are out of "standard" cows.

To the authors the most plausible hypothesis for these ratios of "compact" to "standard" would assume the "compact" type to be due to a single dominant gene. If this is true, under a random assortment of genes the expected numbers of "compact" and "standard" calves would be those in the right-hand columns of Table I. The expected values are those most likely to occur when groups of cows not carrying the "compact" gene and a group in which the frequency of the gene is .29 are mated to heterozygous bulls.

Other evidence for the dominant gene hypothesis is that in previous matings in these herds no "compact" types had ever been obtained from the mating of "standard" type animals. From the matings of "compact" bulls to "compact" cows in the Kuhrt herd only "standard" and "compact" calves have been produced. No more extreme degree of compactness has been noted from "compact" × "compact" matings than from "compact" × "standard" matings.

Show Winnings of the "Compact" Type

The Kuhrt family began showing steers in the National Western Livestock Show at Denver in 1925. Up until 1935 their steers were all of the "standard" type and since that time most of them have been of the "compact" type. A brief summary of their results from showing "standard" type cattle during 1925 to 1934 and "compacts" during 1935 to 1943 is shown in Table II. From this it appears that they have had more lightweights to show and have been more successful with lightweight show steers since using the "compact" type.

Steers purchased from the Crews and Kuhrt herds have been successfully shown by other exhibitors. In the carcass contest at the Fort Worth Stock Show in 1940, the first- ninth- and tenth-placing carcasses in interbreed competition with 250 carcasses of all breeds were "compact" steers bred by the Kuhrts. As yet little is known about the weights and economy of gains of

TABLE I. Actual and expected numbers of "compact" and "standard" type Shorthorn calves sired by "compact" bulls in three purebred herds.

Herd	Dam's Type	Type of Calves			
		Actual Number		Expected Number	
		Compact	Standard	Compact	Standard
Crews	All "standard"	43	31	37	37
Lindgren	All "standard"	21	17	19	19
Kuhrt*	27 "compact" / 20 "standard"	24	18	27	15

*Five cows classified in the Kuhrt herd did not have calves, but which five was not known at the time of inspection in the large pastures; so all were included in calculating the frequency of the "compact" gene in this group of dams.

TABLE II. Number of Kuhrt steers that have been in the first six placings at the National Western Stock Show.

	WEIGHT DIVISIONS				
	Up to 875 lbs.	875 to 1,000 lbs.	1,000 to 1,130 lbs.	1,130 to 1,300 lbs.	Group of 3 steers
"Standards"* 1925-1934	4	10	5	5	6
"Compacts" 1935-1943	14	9	6	2	8

*None shown in 1930.

the "compact" type as compared with the "standard" type. The calves pictured in Figure 12 are on digestion trials at the Colorado Agricultural Experiment Station, Colorado State College. These pairs of steer calves are considered to be representative of the two types found in the three herds. Within each pair there is not more than a week's difference in age. The picture was taken when the calves were in pasture conditions and about six months old.

Summary

An extreme degree of compactness of body and shortness of bone has been observed in three purebred Shorthorn herds. Ratios found in unselected calf crops indicate that the character may be due to a single dominant gene. It is not known as yet whether this gene may be similar to the "duck-legged" gene reported in Hereford cattle (Lush, 1930), or the "bulldog" gene found in Dexter cattle.

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Cancer Histories of "Predisposed" Sibships

Hanhart (*Schw. Med. Wosch.* 73:446) reports a study of descendants of 121 married couples in which both partners had cancer. A total number of 590 children sprang from these marriages, 359 of whom lived to be over 40 years of age, 286 over 50 and 188 over 60. Only 30 of the 121 couples had children who developed cancer. The total number of the children was 43, 26 men and 17 women. Two of them developed cancer in their forties, 3 in their 50 fifties, 11 in their sixties and 27 in their seventies. Except for a few, all died from cancer. Only one-seventh of the 188 oped cancer likewise, whereas six-sevenths of children who lived to their seventies and both the persons did not at the age which the of whose parents suffered from cancer develhazard of cancer is greatest in Switzerland. The average age to which both partners of the 121 parents attained was 66 years. The average of the 30 couples whose children developed cancer was 68 years. The average age to which their children with cancer lived was 61 years. It cannot be concluded from this limited material that the descendants may develop cancer at an earlier age than their parents. A single case may occasionally be significant in some other direction. Two children, a son of 65 and a daughter of 60 years, were in good health although their father had primary multiple malignant epitheliomas and their mother died from cancer of the uterus. The series of 121 married couples with cancer represented the incidence in the canton of Glarus. The average cancer expectancy of their 286 children when past 50 years of age amounted to around 13 per cent, whereas cancer expectancy of the population of the city of Zurich (about 320,000 people) was about 20 per cent. Both parents of the 33 out of the 121 married couples developed cancer of the stomach, and only 11 per cent of their children did likewise. Thus Julius Bauer's concept of a special predisposition of certain organs to cancer was not demonstrated. The results of this study are much like those of investigations on twins. They suggest absence of a specific hereditary predisposition to cancer.—*J.A.M.A.* 125:826.

QUINTUPLE LAMBS OF WOOLLESS DOMESTIC SHEEP

DAVID H. JOHNSON
U. S. National Museum

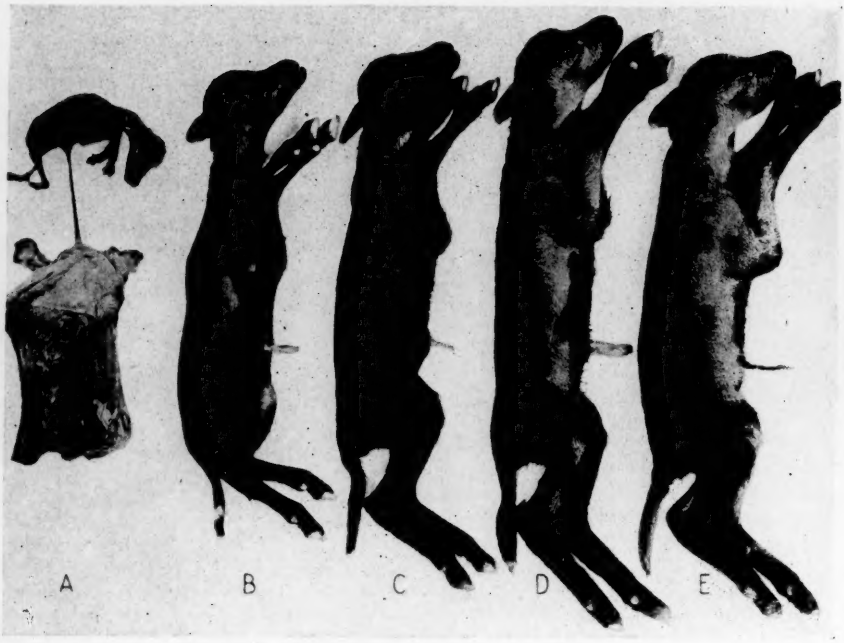
TWINS and triplets occur as rather frequent exceptions to the usual single lamb per birth in domestic sheep (*Ovis aries*), but a "litter" in excess of three is exceedingly unusual in the common breeds. The more prolific woolless type known in America as "Barbados" sheep has interested breeders as providing a possible means of increasing the number of offspring of mutton and wool producing sheep through cross-breeding. However, experiments along this line have proved unsatisfactory, and the woolless type is now best known as an infrequent exhibit in zoological gardens.

On April 28, 1943, five stillborn lambs were dropped by the ewe of a pair of these sheep in the National Zoological Park at Washington, D. C. The accompanying photograph (Figure 13) shows the condition and relative sizes of the five lambs. The four apparently normal individuals include a single male (*B*) and three females (*C, D, E*). The aborted and shrunken individual (*A*), shown with part of the placenta attached, was a female that had apparently been dead for some time before birth. This fetus and its umbilical cord with the adjacent part of the placenta, including several placentary cotyledons, were hardened and brownish-gray in color, in sharp contrast to the normal reddish color of the unaffected placenta. The boundary of the affected area is clearly shown in the photograph. Umbilical blood vessels from different lambs were distributed in common to the various cotyledons, and it seems possible that the four normal lambs were killed by substances diffusing into the common fetal circulatory system from the dead individual.

The five lambs are preserved in the United States National Museum (nos. 271013-271017). Their weights (in grams) and the four external measurements commonly used by systematists (in millimeters) are as follows:

	Sex	Weight	length	Length of tail	Length of hind foot	Length of ear
a	♀	71	165	19	45	14
b	♂	681	444	82	111	41
c	♀	695	479	106	119	43
d	♀	908	509	101	134	46
e	♀	880	500	105	130	43

According to Shaw and Haller[5] the United States Department of Agriculture imported a ram and four ewes of this breed from the West Indian island of Barbados in 1904. In the flock derived from this group, twins and triplets exceeded "singles," and on one occasion six lambs were born. As a contrast, the experiments of Alexander Graham Bell, in which he attempted to increase by selection the number of offspring in a flock of ordinary sheep, are of interest here. Of 565 births recorded by Bell,[1] only 89, or 15.8 per cent, produced twins and there was only one set of triplets. Castle,[2] summarizing Bell's 34-year selection experiment found that in the strain used by Bell multiple birth tendencies were little affected by selection. On August 24, 1942, eight months before the quintuplets were born, the pair in the National Zoological Park produced a single male lamb. The parents of the quintuplets are still on exhibition, and the Assistant Director of the National Zoological Park, Mr. Ernest P. Walker, has kindly furnished photographs of them (Figure 14). Nothing is known of their history except that they were purchased from an animal dealer in 1942. The original home of the breed is probably West Africa, where native tribes are known to keep sheep of a variety of types, including generalized short-haired sheep answering the description of those in question (see



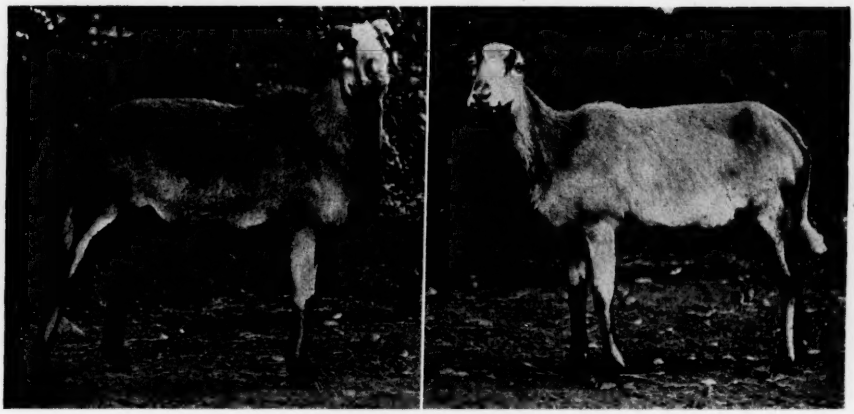
QUINTUPLET WOOLLESS SHEEP

Figure 13

One of the lambs (*B*) is a male, so that the set is not identical. One of the quints (*A*) died a considerable time before birth. Possibly toxic substances from this dead fetus was fatal to the others, as all were born dead.

Lydekker,[4] pp. 212-244, and Hilzheimer,[3] pp. 257-260). In size and general proportions they resemble the larger familiar domestic breeds, although they differ from what is considered a desirable mutton type in being far too narrow-bodied, long-necked, and angular. They are fairly long-legged, but not to the extreme degree exhibited by some of the other African types. The ears do not droop, and the dorsal outline of the muzzle is not conspicuously convex. There is no unusual deposit of fat on the rump or tail. The male has relatively small bluish-gray horns that describe only about half a spiral and are closely appressed to the sides of the head; the female is hornless. The tail reaches to the hocks. The hair of the body averages about an inch long and in texture resembles that of a domestic goat. There is a fringe of longer hair running down the mid line of the throat, and on the male there is a shaggy mane of hair four to six inches long extending from the bases of the horns to the shoulders.

The peculiar color pattern is of the "reversed" or "black-bellied" type in which the underparts, instead of being paler than the upper parts, are black. A similar pattern is also found in various wild animals, either as a sporadically appearing "color phase" (as in the fox squirrel, *Sciurus niger*) or as a feature of an entire population (the Kaibab squirrel, *Sciurus kaibabensis*). The black markings on the sheep cover the lower jaw, throat, breast, belly, axillary and inguinal regions, and inner sides of the legs, and extend as a narrow line along the underside of the tail nearly to the tip. On the outer side of each leg



WOOLLESS SHEEP

Figure 14

Resembling the larger breeds in general conformation, these "Barbadoes sheep" are covered wtih hair instead of wool. A tendency to have a greater frequency of multiple births than in the wooled breeds has been the basis for several cross-breeding experiments. None of these has been marked by success.

the paler dorsal color persists only as a restricted and more or less broken stripe. The inner (concave) surface of the ear is black, and there is a conspicuous black stripe on the face above and anterior to each eye. In the adult male the occipital area immediately behind the horn bases is black. Where the hair is short, as on the breast and belly, the black area is sharply delimited, but in the longer hair of the outer sides of the thighs and on the mane of the male the transition from black to pale color is more gradual. The color of the back and sides is a rich reddish brown, which becomes paler on the face, the sides of the neck, and the flanks. The tip of the tail is white, although this feature is somewhat variable, as is shown in the photograph of the lambs.

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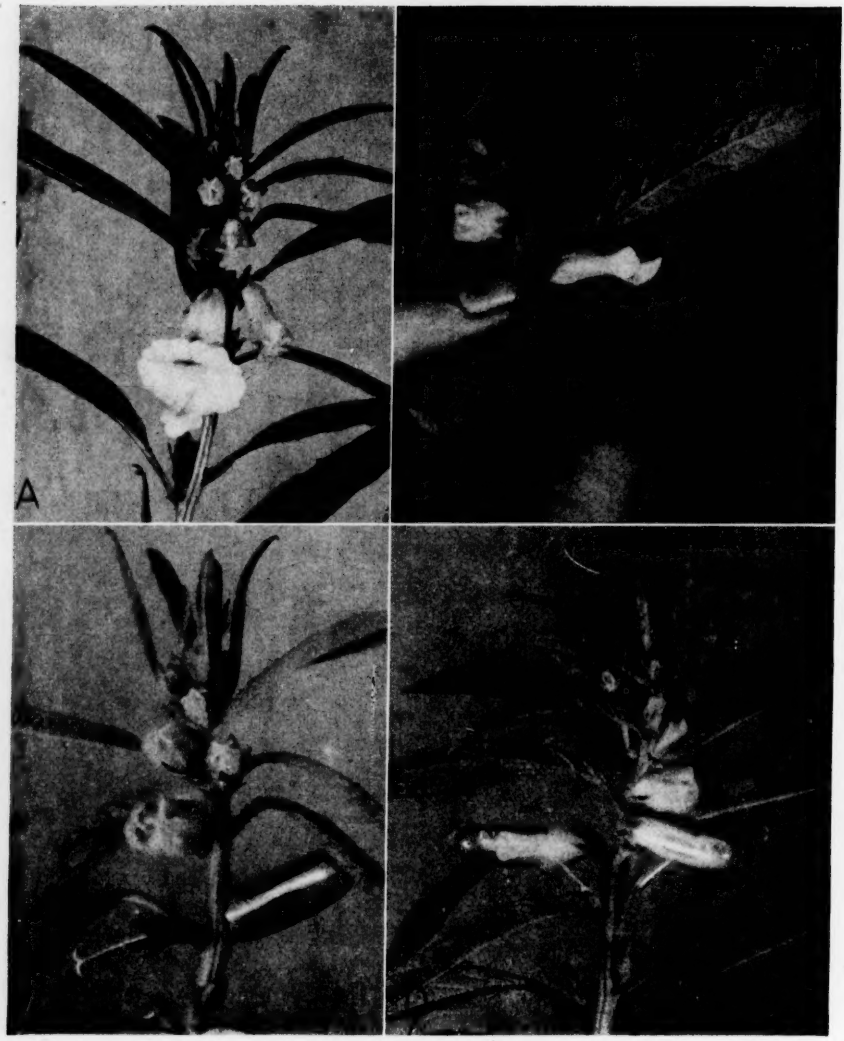
✦

Giving Man a "Break"

A conception of the full utilization of human and material resources for the benefit of man is just breaking in upon us. Everyone expresses it somewhat vaguely and no one quite foresees all the possibilities. Science and invention have given us the modern world through the use and development of material resources, they have not yet given equivalent use and full development of human resources. The psychologist has a significant scientific and practical role; he knows more about man as a person than anyone else—it remains for him to broaden and deepen his conception of the environment by a thorough-going analysis. Let us hope that with the war out of way, that we will have the opportunity, the energy, and the understanding to play our roles in the "years of the modern, years of the unperformed."—ANDERSON, JOHN E. *Psych. Bull.* 111:27.



POLLINATION OF SESAME

Figure 15

A shows the pendulant flowers, one at the axil of each leaf. Flowers used for cross pollination must be emasculated before they are open. This is accomplished (*B*) by holding the bottom part of the corolla and jerking it quickly, which removes the unopened anthers as well. The adjoining leaf is pinched off and a gelatine capsule is placed over emasculated flower and leaf petiole. The flowers to be used as male parents are also covered with capsules (*D*), to prevent contamination. By this method of crossing, seed production is not materially less than in normally open-pollinated flowers.

NATURAL AND CONTROLLED POLLINATION IN SESAME

D. G. LANGHAM

Instituto Experimental de Agricultura y Zootecnia, Caracas, Venezuela

SESAME (*Sesamum* indicum L.) has been an important source of vegetable oil at least since early Egyptian times and is rapidly becoming an important crop plant in several of the Latin American countries. As is to be expected of a crop of such antiquity, there are many varieties. A study of a number of these from Venezuela, Colombia, Guatemala, Honduras, Mexico, Nicaragua, Brazil, India and China shows that among them are many desirable characters. The need of developing improved varieties adapted to the conditions of Venezuela by combining the best characters of several varieties, has led to a rapid method of making artificial pollinations. This technique was developed at our station after many methods had been tested during a period of two years. Although developed entirely independently, it closely resembles the "soda straw" technique described by Doak* for cotton hybridization, using gelatine capsules instead of soda straws. The essential features of the method are shown in Figure 15.

The flowers of sesame are ordinarily solitary in the axils of the leaves and pendulent the day before they open (Figure 15*A*). For cross-pollination the flower must be emasculated before it opens. A quick jerk on the inferior part of the corolla with the thumb and forefinger (Figure 15*B*) is sufficient to remove it with the complement of unopened anthers attached to the corolla wall. The ovary, style, and stigma are left intact. The leaf is then pinched off the same length as the pistil, to serve as a support for the gelatine capsule and as a source of humidity for the exposed stigma. A gelatine capsule, size 0 or 00 as fits best, is then slipped over the shortened leaf and the pistil (Figure 15*C*). Another gelatine capsule is used to cover the corolla of the variety chosen as a male parent (Figure 15*D*). (When mass crosses between two varieties are to be made, many unopened corollas with their stamens may be collected from the male parent and placed in a moist chamber over night.)

The following morning the corolla containing the dehisced anthers of the protected flower of the male parent is removed by a quick jerk as described above (Figure 15*B*). If the open end of this corolla is clipped off, exposing the pollen-laden anthers, the pollination may be performed by placing the anthers in direct contact with the stigma of the female parent. A slight twirl of the corolla between the fingers insures a thorough dusting of the stigma. The gelatine capsule is returned to the pollinated stigma and is supported by the leaf petiole. In a few days the capsule dissolves in the rain or is pushed off the seed pod by the growing leaf petiole. A string tag in the axil of the leaf marks the cross.

Self-pollination can be made by covering the entire flower the day before it opens with a gelatine capsule (Figure 15*D*) to protect it from bees and other insects. The following morning the corolla is removed from this flower, and the attached anthers are used to dust the stigma of the same flower. The leaf is then pinched off the same length as the pistil so that it supports the gelatine capsule. A string tag marks the flower.

Many thousands of controlled pollinations have recently been made by the above method. Nearly all of them were successful. The number of seeds per pod obtained by artificial pollination is essentially the same as the normal complement.

The necessity of protecting the stigma

*DOAK, C. C. *Journal of Heredity* 25:201-204. 1934.

of the emasculated flowers from foreign pollen was demonstrated by emasculating 81 flowers and leaving the stigmas exposed. Seventy-one of these dropped, but ten of them, or 12.5 per cent, developed a few seeds.

The effectiveness of the gelatine capsule in protecting the stigma from foreign pollen was demonstrated by emasculating 96 flowers and covering them with capsules as described above. No pollen was applied, and none of the flowers set seed.

Natural Cross-Pollination In Sesame

In a field of approximately three thousand sesame plants, four were found which differed from all the others in being glabrous, with practically no pubescence and few glandular hairs. Some flowers of each of these plants were artificially self-pollinated and some were crossed with non-glabrous plants; but the majority of the flowers were open-pollinated.

The seeds from self-pollinated flowers of each of the four plants gave glabrous seedlings. Those from the cross-pollinated flowers gave non-glabrous seedlings, demonstrating that non-glabrous is dominant over glabrous. The seeds from the open-pollinated flowers, then, should be useful for determining the per cent of cross-pollination occurring naturally in sesame.

Accordingly, the seeds from the open-pollinated flowers were planted in flats and one of the first true leaves from each seedling was examined to see whether it was glabrous like the parent plant, or non-glabrous, indicating natural cross-pollination. It was found that by removing one leaf from each plant and placing the group of leaves near a window, with the lower side of each leaf upward, the glabrous ones could be distinguished from the non-glabrous ones with the naked eye. The results of the test are given in Table I.

Due to the abundance of honey bees seen visiting the sesame flowers, it is believed that they are largely responsible for the 4.6 per cent cross-pollination observed in this experiment. These bees alight on the protruding lip of the flower and squeeze inside. They emerge with their pollen baskets full and with a considerable number of pollen grains adhering to their bodies, and immediately visit another flower. A small fly also visits the sesame flower occasionally.

Summary

1. A simple method of making controlled pollinations in sesame is described.

2. Under the field conditions 4.6 per cent of cross-pollination was found, attributed to visits of insects, mostly honey bees.

TABLE I. Frequency of self-pollinated (glabrous) and cross-pollinated (hairy) seedlings in sesame with open pollination under field conditions.

Plant No.	Phenotype	Phenotype of Seedlings: Number and Per Cent Glabrous	Not Glabrous
1	Glabrous	695—98%	14—2%
2	Glabrous	325—94.2%	20—5.8%
3	Glabrous	860—94.3%	52—5.7%
4	Glabrous	1133—94.2%	61—5.8%
		3013—95.4%	147—4.6%

✦

Bricklayers Without Blueprints

In the words of the old cliche, in fact, scientists have tended to refuse to see the wood for the trees. They have been an army of bricklayers, piling brick on brick, even plumbers setting up super W. C.'s, and heating and lighting engineers installing the most modern equipment; but they have all united to shoo the architect off the building site, and the edifice of knowledge is growing like a factory with a furnace too big for its boilers, its precision tools installed in a room with no lighting, and anyhow with no one who knows what it is supposed to manufacture. —C. H. Waddington. *The Scientific Attitude,* P. 63.

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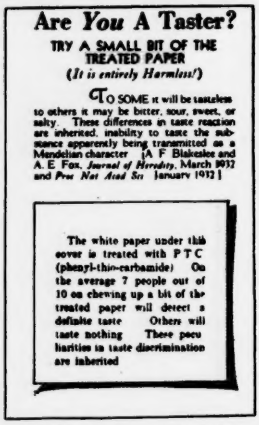
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